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Benefits and costs of increased levels of corticosterone in seabird chicks

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Abstract

Seabird chicks respond to food shortages by increasing corticosterone (cort) secretion, which is probably associated with fitness benefits and costs. To examine this, we experimentally increased levels of circulating cort in captive black-legged kittiwake chicks fed ad libitum. We found that cort-implanted chicks begged more frequently and were more aggressive compared to controls. These behavioral modifications must be beneficial to chicks as they facilitate acquisition of food from the parents and might trigger brood reduction and reduced competition for food. Cort-implanted chicks also increased food intake; however, their growth rates were similar to controls. To examine the costs of chronically increased circulating levels of cort, we removed cort implants and, after a 10-day recovery period, tested cognitive abilities of young kittiwakes. We found that the ability of kittiwakes to associate a visual cue with the presence of food in a choice situation was compromised by the experimental elevation of cort during development. To examine the long-term costs of increased levels of cort, 8 months later we tested the performance of the same individuals in a spatial task requiring them to make a detour around a barrier in order to escape from an enclosure. Individuals treated with cort during development took significantly more time to solve this task compared to controls. The results of this study suggest that the adrenocortical response of a developing bird to environmental stressors is associated with both benefits (increased food intake, foraging behavior, and aggression) and costs (low growth efficiency and compromised cognitive abilities later in life). This provides an evolutionary framework for relating juvenile physiological traits to fitness of birds in subsequent life-history stages.

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Introduction

Nest-bound chicks of several species of seabirds respond to food shortages by increasing corticosterone (cort) secretion (e.g., blue-footed boobies, *Sula nebouxii*, Nuñez-de la Mora et al., 1996; black-legged kittiwakes, *Rissa tridactyla*, Kitaysky et al., 1999; red-legged kittiwakes, *Rissa brevirostris*, Kitaysky et al., 2001a; common murre, *Uria aalge*, Kitaysky et al., unpublished data). Increased levels of cort are probably associated with fitness benefits and costs that must be balanced. Short-term release of cort is beneficial to hungry black-legged kittiwake chicks because it allows

them to restore energy reserves by increasing begging for food and thereby increasing parental food provisioning (Kitaysky et al., 2001b). If parents are not able to increase energy delivery to the chicks, elevated cort secretion becomes chronic (Kitaysky et al., 2001a). Chronic elevation of stress hormones has been shown to be detrimental in many species (reviewed in Sapolsky et al., 2000), but whether this applies to juvenile birds is largely unknown. A better understanding of fitness benefits and costs of cort release in chicks would provide insight into evolutionary factors shaping juvenile physiological and/or behavioral traits, which determine fitness in subsequent life-history stages.

Chronic elevation of stress hormones affects cognition in mammals (Sapolsky, 1992; Sapolsky et al., 1986; recent review in Sapolsky et al., 2000). In other taxa, inferior

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cognitive abilities have been shown to reduce reproductive fitness (Dukas and Bernays, 2000), and to affect behavioral traits that are highly correlated with fitness (e.g., Nowicki et al., 2000). While it seems intuitive that exposure to chronically elevated levels of cort during early development might affect learning in birds, to our best knowledge there are no experimental data that directly show this. Experimentally induced nutritional stress in red-legged kittiwake chicks results in chronically elevated levels of cort (Kitaysky et al., 2001a), and cognitive abilities of individuals that were nutritionally stressed during early development are severely compromised (Kitaysky et al., unpublished data). However, whether cognition was affected by temporary developmental retardation, by chronic elevation of cort, or a combination of factors resulting from nutritional stress remains unknown.

In this study we examined benefits and costs of chronic elevation of cort in black-legged kittiwake chicks. Black-legged kittiwakes are colonial, cliff-nesting gulls with a maximal brood size of three. A nutritionally stressed kittiwake chick appears to have only two behavioral options to improve its chances of survival: either to eliminate nest mates (perform siblicide) or to increase its begging for food (Braun and Hunt, 1983). In an earlier field study (Kitaysky et al., 2001b), we showed that experimentally elevated levels of cort facilitate begging but did not increase aggression of chicks. Our objectives in the current study were to test the effects of experimentally increased levels of cort (dissociated from nutritional stress / developmental retardation) on (1) aggression of naïve chicks (without prior social contact with other individuals) and (2) efficiency of growth; and to test the hypothesis that prolonged elevation of cort during early development compromises cognition of birds later in life.

Methods

Subjects

Partially incubated eggs of free-living black-legged kittiwakes were collected on Gull Island colonies (Lower Cook Inlet, Alaska) and transported to the University of Washington facilities. During egg collection, one egg from a nest (containing two or three egg clutches) was taken randomly. Eggs were incubated using Lyons incubators at 37.2°C and 56% relative humidity. A total of 21 chicks were raised in individual nests in a 12d:12l photoperiod, at 30°C and 80% relative humidity (newly hatched to 7 days post-hatch); at 25°C and 70% relative humidity (7–25 days post-hatch); and at outdoor ambient temperatures, humidity, and photoperiod thereafter. All chicks were kept in the same room in physical, but not in visual and/or acoustic isolation from each other. Kittiwakes were fed fish high in nutrients, capelin *Mallotus villosus*, (with two tablets of multivitamin/mineral supplement on daily basis) given ad libitum during

the entire experiment. From hatching chicks were fed only after they started begging (frequent vertical movements of the head accompanied by a high-pitch vocalization) and pecking at the beak of a puppet (shaped and colored similar to the head of an adult kittiwake). For individual identification all birds were banded with a unique combination of leg color bands. After fledging (at 43 days of age), kittiwakes remained in the same aviary where they had been raised since 25 days post-hatch. During their time in the facilities of the University of Washington, kittiwakes were exposed to the same personnel and their contact with other people was limited. When birds were 11 months old, they were donated to the Rotterdam Zoo (Netherlands) for captive breeding programs and permanent exhibition.

Cort treatment

Upon hatching each chick was randomly assigned to one of three treatments. When chicks were 14 days post-hatch, they were implanted subcutaneously with two 25-mm silastic tubes (Dow Corning) that were either filled with crystallized cort or left empty. In two cort treatments (8 and 7 individuals, respectively) chicks were implanted with either one cort implant (second implant was empty) or two cort implants, whereas controls (6 individuals) were implanted with two empty implants. Blood samples were collected on a weekly basis (0, 7, and 14 days after implantation) from undistributed post-absorptive (after overnight fast) individuals by puncturing the alar vein and collecting blood in heparinized microhematocrit 10- μ l tubes. After blood collection, hematocrit tubes were emptied into 0.5-ml vials, which were stored at 4°C. Within 6 h samples were centrifuged and plasma was collected. Plasma samples were frozen at –20°C until radioimmunoassay (for detailed protocol see Wingfield and Farner, 1975, and Wingfield et al., 1992). Cort concentrations were measured in duplicate for each plasma sample in a single assay after extraction in dichloromethane. Before extraction, tritiated cort (2000 cpm) was added to each sample to control for loss of cort during extraction. Recovery values of the labeled steroid ranged from 85 to 95% and were used to adjust assayed concentrations of cort. Sensitivity of the assay was 7.6 pg/tube.

Four weeks after the beginning of cort treatments (on Day 42 post-hatch), subcutaneous implants were removed from all birds. Upon removal, cort implants retained approximately 2/3 of the initial volume of steroid. We did not observe any signs of implant encapsulation by a bird's mesenteric tissue.

Measurements of skeletal elements, body mass, and food intake

Body mass of post-absorptive chicks was measured every 5 days. Bill length (culmen), tarsus, and wing length were also measured every 5 days. Daily food intake of chicks was measured on a weekly basis (0, 7, 14, and 21

days after implantation). Food was offered to all chicks ad libitum four times per day, and the exact amount of food eaten by each chick was recorded. Food intake was not measured at the end of the last week (Day 28) of cort treatments because birds started switching their nests, which made precise measurement of the food intake impossible.

Aggression experiment

At age 21 days post-hatch (a week after the beginning of cort treatments) behavior of chicks in relation to cort levels was tested. Two naïve chicks (no previous experience of physical contact with each other, or any other chick) were placed in an experimental arena (similar in size and structure to individual nests where chicks had been raised) established in a room familiar to both chicks. The experimental arena was elevated 1 m above the floor. A puppet imitating the head of an adult kittiwake (see above) was hanging from the ceiling of the arena as a food stimulus. The room was visually and acoustically isolated from the aviary with other chicks. Chicks were post-absorptive. Within each pair ($n = 6$), chicks were of the same age, but one was implanted with a single cort implant and the other was sham-implanted. For visual identification each chick was marked with a small piece of white sticky tape (with identification number) on the top of its head. Behavior of chicks was continuously recorded during a 60-min period through a one-way mirror. Number of aggressive attacks and duration of begging were recorded. Begging was defined as frequent vertical movements of the head and pecking on the “beak” of the puppet. Aggression was defined as a chick pecking / grabbing / twisting skin on the head/nape of the other chick. All observations were carried out by the same person, who was blind to chicks’ treatments.

Cognition experiment 1

Animals’ motivation/willingness to cooperate during experiments is important for studies of learning behavior. Hand-rearing guaranteed that the kittiwakes in this experiment were tame and cooperative. During training all birds were habituated to the experimental conditions until they showed no visible signs of fear and/or anxiety. An isolated kittiwake behaved calmly only if it was able to hear the rest of the birds. Thus, during experiments, the focal bird was visually but not acoustically isolated from the rest of the group.

Apparatus

The experimental room (2.4 m long \times 1.8 m wide \times 2 m high) was adjacent to the common aviary. Walls of the room were covered with black tarpaulin to visually isolate experimental animals from an observer and the rest of the group. Transparent and solid-colored dishes used during experiments (see below) were identical plastic cylinders 9.5 cm in diameter and 1.8 cm in height. Dishes identical in color and

size were used as lids and bottoms. Dishes were placed (20 cm apart) in a chessboard pattern and locations of dishes were fixed during experiments. Dishes were all smeared with fish on the lid to control for possible effects of odor.

Procedure

For details and procedures of experimental design see Table 1.

Habituation and training. During training (between Days 44 and 51 post-hatch), all birds were habituated to being picked up by an observer, placed alone in the experimental room, and eating from a transparent dish (with no lid) placed on the floor in the center of experimental room. Experimental trials started when kittiwakes were 52 days old (10 days after cort implants were removed). During each trial a post-absorptive chick was placed in the experimental room with dishes. First, birds learned to open (by pushing off the lid) a closed transparent dish containing food. Then, the presence of food in the dish was associated with the color of a solid-colored opaque dish: black dishes contained food (a single 0.5-g piece of capelin in each dish) and white dishes were empty. Kittiwakes exhibited no preference for either color during the first trial with two (one black and one white) solid-colored dishes (9 individuals first opened a black dish and 10 individuals first opened a white dish). The number of solid-colored dishes (with equal number of dishes with and without food) increased (2, 4, 6, and 12 dishes) in each of four consecutive trials. During the last trial of the learning stage of the experiment, we repeated the 12-dish trial (see Table 1).

Testing. Each bird participated in an experiment on a daily basis, but no more than a single 10-min trial per day was performed on the same individual, except for the first two trials with transparent closed dishes containing food (Table 1). In particular, individuals that did not open a dish during the first 10 min were left in the experimental room until they finished the task, or for a maximum of 60 min. If an individual did not open a single transparent dish with food (Trial 1, see Table 1) after three consecutive 60-min trials (conducted during 3 consecutive days), it was excluded from the experiment.

To test whether kittiwakes memorized the task, we kept birds in the common aviary without exposure to dishes for 2 weeks after the last learning trial and then ran the 12-dish trial again. Finally, because the location of dishes was fixed, birds could have memorized the location of dishes with food rather than have learned to associate the color with dish content. To examine this possibility we changed the position of dishes in an inverse pattern and repeated the 12-dish trial.

We conducted all observations through a one-way mirror to prevent an observer effect. The same observer conducted trials on all birds. Time was recorded immediately after a bird was placed in the experimental room. Behavior

Table 1
Experimental protocol used to test cognitive abilities of young black-legged kittiwakes

	Description, timing, and complexity of experimental trials								
	Trial 1	Trial 2	Trial 3	Trial 4	Trial 5	Trial 6	Trial 7	Trial 8	Trial 9
	Training to open a dish		Training to associate visual cue (color) with presence of food			Testing ability to associate visual cue with presence of food		Testing memory ^a	Testing effects of dish placement ^b
	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 21	Day 22
Transparent dishes with food	1	2							
Dishes with food (black)			1	2	3	6	6	6	6
Dishes without food (white)			1	2	3	6	6	6	6
Dishes total	1	2	2	4	6	12	12	12	12

^a Trials 7 and 8 were conducted 2 weeks apart.

^b Testing whether birds memorized locations (rather than color) of dishes with food (for details see Methods).

of a bird, such as time spent opening a dish (between first peck on a dish and eating food), total number of dishes opened, and sequence in which a bird opened dishes was continuously recorded. During trials when birds opened multiple dishes, the time taken by a bird to open the first dish (between first peck on dish and eating food) was used in the analyses. The success of a bird in learning to associate the color of a dish with the dish content during the 12-dish trials (Table 1) was calculated as a proportion of correct choices made among the first 6 opened dishes.

Cognition experiment 2

To examine the long-term effects of cort treatment on cognition of kittiwakes, the same individuals were tested at age 10 months post-hatch (9 months after cort treatments). Three kittiwakes that were subject to elevated cort treatments during development (one individual from the single cort implant treatment and two individuals from the two cort implants treatment) died of systemic infection at age 4–6 months post-hatch; all other individuals appeared in healthy condition.

Apparatus

In this study we used a modified version of an apparatus and a general approach previously determined to be appropriate for testing long-term memory and learning of a spatial task in domestic fowl chicks (Regolin and Rose, 1999). Fig. 1 shows the experimental arena (hereafter called “enclosure”). A large squared enclosure (100 cm wide \times 40 cm deep), with its roof and floor made of chicken wire and walls (30 cm high) made of plywood, was established on the middle of large outdoor aviary (5.4 m long \times 2 m wide \times 2.4 m high). Each wall of the enclosure had an opening shaped as a corridor (20 cm wide \times 40 cm deep \times 30 cm high). All but two openings on opposite sides of the enclosure were permanently closed with transparent doors also made of chicken wire. A door (20 cm \times 30 cm) located in the center of the roof was used to place subjects in the

enclosure. The aviary with the enclosure was adjacent to the common aviary where all kittiwakes were permanently housed and it was visually but not acoustically isolated from the common aviary. All birds showed signs of fear and anxiety while in the experimental arena and actively tried to escape the enclosure. After exiting the enclosure, individuals were immediately transferred back to the common aviary.

Procedure

Post-absorptive kittiwakes were individually placed in the center of the experimental arena. On the first trial, each bird went directly to the closed opening (opening A, Fig. 1) facing the common aviary and tried to get through the transparent door. Birds then searched for other routes to escape the enclosure, and eventually found one of the two open corridors and used it to exit. The time each individual took to escape the enclosure (time recording started immediately after placing a bird in the enclosure) and the opening used by each bird to exit the enclosure were recorded. Each individual was tested in a single trial on each of the three consecutive days. Each individual consistently (in all three

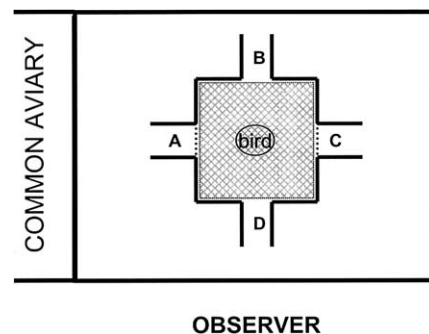


Fig. 1. The apparatus used in experiment 2. See text for description. Openings A and C were permanently closed with a transparent door. Openings B and D were opened during the first three trials. The opening (either B or D) that a particular bird habitually used to escape the enclosure was closed during the fourth trial.

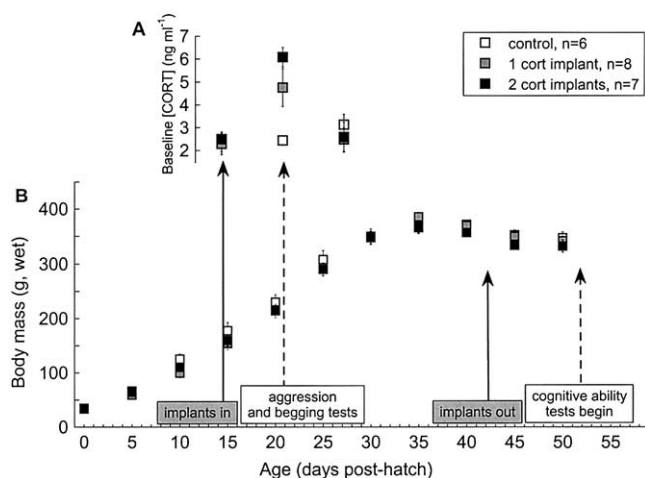


Fig. 2. Baseline plasma levels of corticosterone of black-legged kittiwake chicks in relation to duration of the corticosterone (cort) treatment (0, 7, and 14 days postimplantation; means \pm SE; top panel A). Arrows indicate corresponding age of the chicks. Body mass (means \pm SE) of black-legged kittiwakes in relation to their age and cort treatments (bottom panel B). The figure also shows timing of implantation and implant removal and timing of aggression and learning experiments.

consecutive trials) used the same opening (either B or D), although different individuals used different openings. On the fourth day of the experiment, the opening that an individual bird always used to exit the enclosure was closed (with a transparent door made of chicken wire) and the time taken by that bird to locate the other opening was recorded. All trials were conducted during morning hours. The same observer conducted trials on all birds; observations were made through a one-way mirror.

Statistical analyses

All computations were performed with Systat and Statistica statistical packages. During statistical analyses, initial data were tested for assumptions required by statistical tests according to Sokal and Rohlf (1981). If data violated the assumptions for parametric tests they were examined with nonparametric equivalents. Statistical significance was assumed at P (two-tailed) < 0.05 . The effects of cort treatments on baseline levels of cort, growth parameters, and food intake were examined with repeated measures ANOVAs (followed by post hoc LSD planned comparison tests), where treatment was used as a factor and duration of treatment as a repeated measure. The effects of cort on aggression and begging were examined with Wilcoxon matched-pairs tests. The effects of cort treatment on cognitive abilities were tested using repeated measures ANOVAs (followed by post hoc LSD planned comparison tests), where treatment during development was used as a factor and consecutive trials as a repeated measure.

Results

Effects of implantation on baseline levels of cort

Subcutaneous implants elevated baseline levels of cort in kittiwake chicks during the first 7 days of the treatment (Fig. 2A). Cort levels changed significantly between Days 0 and 14 of the treatment (treatment duration effect: $F_{2,36} = 11.75$, $P < 0.0001$). Temporal dynamics of cort concentration were also significantly different among the treatments (treatment \times duration interaction term: $F_{4,36} = 8.51$, $P = 0.008$). Specifically, baseline levels of cort were not significantly different among the treatments prior to implantation (LSD P values > 0.7 ; Fig. 2A) and were significantly higher in chicks subject to single- and two-cort implants compared to controls on Day 7 ($P = 0.005$ and $P < 0.001$, respectively), but were not significantly different among the treatments on Day 14 (P values > 0.4).

Effects of cort on growth rates and food intake

Cort implants did not affect growth of chick body mass (treatment effect: $F_{2,18} = 0.45$, $P = 0.645$; Fig. 2B), bill length ($F_{2,18} = 0.072$, $P = 0.931$), tarsus length ($F_{2,18} = 0.802$, $P = 0.464$), or wing length ($F_{2,18} = 1.047$, $P = 0.372$).

Cort treatment significantly increased chick's food intake (treatment effect: $F_{2,18} = 4.06$, $P = 0.035$; Fig. 3). During the period between 0 and 21 days post-implantation, chicks with cort implants consumed on average 15 and 9% (for one- and two-cort implant treatments, respectively) more food than controls. Differences in food intake were significantly different between controls and individuals treated with a single cort implant ($P = 0.011$). Food intake declined at age 35 days post-hatch in controls but was still significantly elevated in one- and two-cort implant treatments

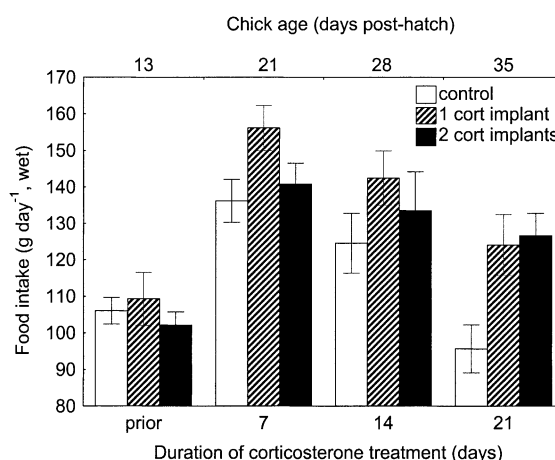


Fig. 3. Effects of corticosterone (cort) treatments on food intake (means \pm SE) of black-legged kittiwakes in relation to their age and duration of the treatment (sample sizes as in Fig. 2).

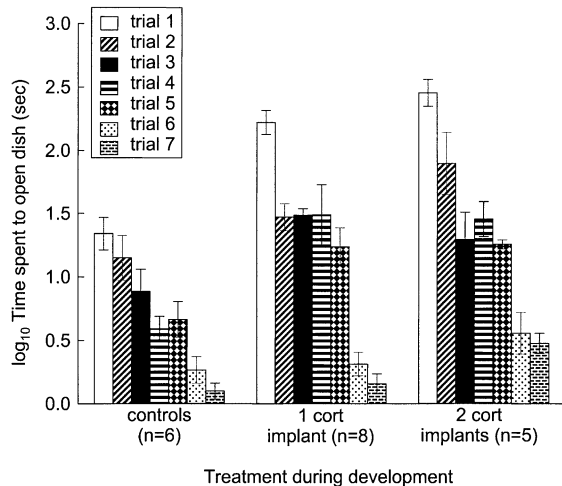


Fig. 4. Effects of corticosterone treatment during development on abilities of young black-legged kittiwakes to learn a new foraging technique—time spent to open a dish (means \pm SE). For description of experimental trials see Table 1 and Methods.

compared to controls ($P = 0.007$ and $P = 0.004$, respectively; Fig. 3).

Effects of cort on aggression and begging

Chicks were examined on Day 7 of cort treatment (21 days post-hatch), when baseline levels of cort were significantly higher in cort-implanted individuals compared to controls (Fig. 2). Cort-implanted individuals attacked the other chick more frequently [5.3 ± 1.89 SE and 0.5 ± 0.34 SE (attacks per trial) for one cort implant and control treatments, respectively; Wilcoxon matched pairs test: $T = 0$, $P < 0.05$, $n = 6$] and spent significantly more time begging from the puppet [367.5 ± 131.72 SE and 22.5 ± 16.63 SE (s of begging per trial) for one cort implant and control treatments, respectively; Wilcoxon matched pairs test: $T = 1$, $P < 0.05$, $n = 6$] compared to controls.

Effects of cort treatment during development on learning to associative learning

Dish opening

Cort treatment during development had a profound effect on a young kittiwake's abilities to learn a "new" foraging technique. Two of seven young birds that had two cort implants during development never learned how to open the single transparent dish with food in the three consecutive training trials (see Methods). Other individuals treated with cort during development took more time during experimental trials to open a dish compared to controls (treatment effect: $F_{2,16} = 21.90$, $P < 0.001$; Fig. 4). Controls took significantly less time to open a dish than individuals subjected to one cort implant ($P = 0.0001$) and two cort implants ($P = 0.00002$), whereas performances of one and two cort implant treatments were not significantly different

($P = 0.151$). All individuals improved their dish-opening skills during consecutive trials ($F_{6,96} = 62.93$, $P < 0.001$), but controls learned the technique significantly faster than the other two treatments (treatment \times number of trials interaction term: $F_{12,96} = 2.54$, $P = 0.011$; Fig. 4).

Associative learning

Cort treatment during development had a significant negative effect on a young kittiwake's abilities to associate color of dishes with the presence of food (Fig. 5). Although kittiwakes in all treatments opened a similar number of dishes during four consecutive 12-dish trials (treatment effect: $F_{2,16} = 3.476$, $P = 0.06$; Fig. 5A), cort-treated individuals made significantly fewer correct choices compared to controls (treatment effect: $F_{2,16} = 7.30$, $P = 0.006$; Fig. 5B). The proportion of correct choices made by controls was significantly higher than that of individuals exposed to one ($P = 0.006$) or two ($P = 0.003$) cort implants during development. Performances of individuals treated with cort were not significantly different between the one-and two-implant treatments ($P = 0.537$). Performance of individuals did not change significantly between trials ($F_{3,48} = 3.03$, $P = 0.061$), and between-trial consistency in performance was similar among the treatments (treatment \times number of trials interaction term: $F_{6,48} = 0.86$, $P = 0.860$).

Trials on memory and on effects of dish placement

Performance of all kittiwakes (regardless of treatment during development) in the memory trial (Trial 8) was not significantly different from their performance in the learning trial (Trial 7) conducted 2 weeks earlier (P values $>$

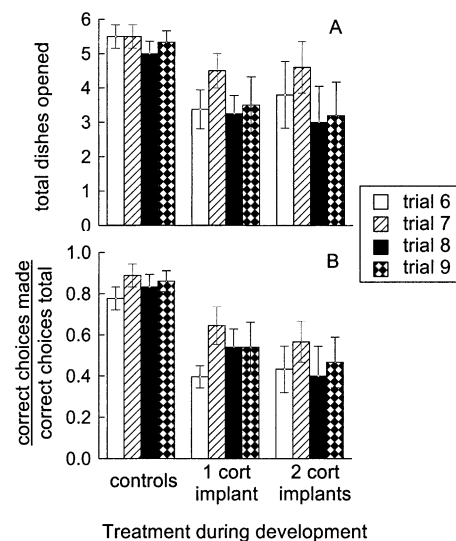


Fig. 5. Effects of corticosterone treatment during development on abilities of young black-legged kittiwakes to associate a visual cue (color of a dish) with dish content. (A) Number (means \pm SE) of dishes (with and without food) opened during experimental trials. (B) Proportion (means \pm SE) of correct choices made (means \pm SE). For description of experimental trials see Table 1 and Methods; sample sizes as in Fig. 4.

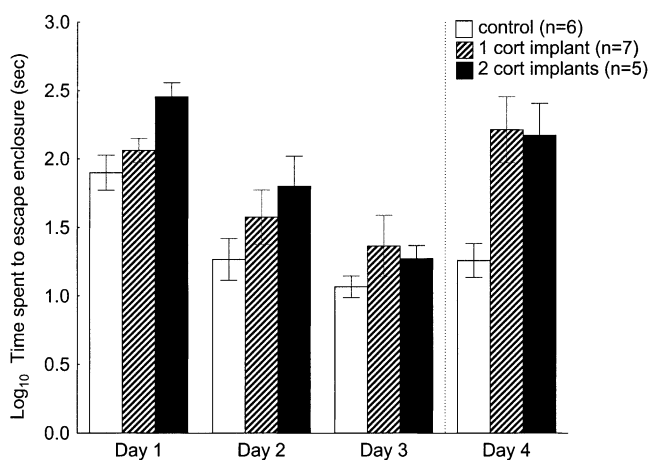


Fig. 6. Long-term effects of corticosterone treatment on abilities of 10-month-old black-legged kittiwakes to solve a spatial task. Figure shows time (means \pm SE) spent by kittiwakes to escape the enclosure. For description of experimental trials see Methods.

0.13; Fig. 5). Also, performance of all individuals in the “inverse placement of dishes” trial was not different from their performance in the memory trial (P values > 0.5 ; Fig. 5).

Long-term effects of cort treatment on solving a spatial task

Cort treatment during development had a significant negative effect on the abilities of 10-month-old kittiwakes to solve a spatial task in order to escape an enclosure (treatment effect: $F_{2,15} = 4.229$, $P = 0.035$; Fig. 6). The performance of all individuals significantly improved over the duration of this experiment (duration of experiment effect: $F_{3,45} = 23.32$, $P < 0.001$), especially during the first three trials (Fig. 6). However, when the preferred opening (that a bird used to exit the enclosure during first three trials) was blocked, cort-treated individuals took more time to locate the other opening than controls (LSD $P < 0.0001$ for both comparisons between controls and individuals treated with single and/or two-cort implants; Fig. 6). Individuals treated with one or two cort implants during development were not significantly different in their performances during the experiment (LSD P s > 0.06 for comparisons of all four trials).

Discussion

Effects of implantation on cort levels

Subcutaneous cort implantation induced a brief twofold increase in concentrations of this hormone in blood of kittiwake chicks. Exogenous levels of cort may be higher during the first 3 days post-implantation (Kitaysky et al., 2001b). The elevated cort concentrations were within the physiological range observed in other captive and field

studies of black-legged kittiwakes (Kitaysky et al., 1999, 2001b). Although the levels of cort were not significantly different between controls and cort-implanted chicks by the end of the second week post-implantation, the implants themselves were only 1/3 depleted of steroid at the end of the fourth week of implantation. We do not know whether this decrease in cort levels was caused by reduced production and/or release of endogenous cort, change in the bound/free ratio of cort, or increased clearance rate of exogenous cort from the peripheral system of experimental chicks. Although cort levels in the blood were not different between controls and experimental individuals by the end of the second week post-implantation, food intake of chicks implanted with cort was still higher by the end of the third week post-implantation. The elevated food intake suggests that exogenous cort had been released and, despite its low concentration in the blood, affected chicks at least during the 3 weeks post-implantation.

High cort and chick aggression and begging

After 1 week post-implantation, chicks with experimentally elevated levels of cort were more aggressive and spent more time begging compared to controls. Facilitation of exaggerated begging by elevated levels of cort has been demonstrated for wild black-legged kittiwake chicks (Kitaysky et al., 2001b). On the other hand, a direct effect of cort on chick aggression in seabird chicks has not previously been shown. Several studies showed that a decrease in parental food provisioning results in sibling aggression (blue-footed boobies, Drummond and Garcia Chavelas, 1989; ospreys, *Pandion haliaetus*, Machmer and Ydenberg, 1998; black guillemots, *Cephus grille*, Cook et al., 2000). An increase in aggression and siblicide by hungry kittiwake chicks has also been demonstrated (e.g., Braun and Hunt, 1983; Irons, 1992). Furthermore, Ramos-Fernandez and co-authors (2000) found that aggression in blue-footed booby chicks coincided with an increased secretion of cort, and suggested that release of cort might directly regulate aggression in seabird chicks. In an earlier study we found that nutritional deficit increases adrenocortical activity of black-legged kittiwakes (Kitaysky et al., 1999). Although experimentally increased circulating levels of cort in wild black-legged kittiwake chicks did not result in an increase in sibling aggression (Kitaysky et al., 2001b), these results were obtained in a situation where the social hierarchy within a brood was established prior to manipulation of the chick's cort levels and in the context of a food-rich colony. In the current study we observed an increase in aggressive behavior in response to experimentally elevated cort in naïve chicks, which might explain the discrepancy. Also, the post-absorptive (after overnight fast) status of chicks could have a permissive effect on stimulation of aggression by high levels of cort.

High cort and chick growth and development

Food intake was higher in individuals exposed to exogenous cort compared to controls. This effect was especially well-pronounced at the end of the third week post-implantation, when controls began a pre fledging recession of food intake, whereas individuals treated with cort continued to eat at a high rate. It is likely that food intake is limited by a chick's physical capacity to ingest some maximal volume of food and by a maximal rate of food processing (reviewed in Ricklefs et al., 1998). At the same time, food intake reflects chick energy requirements, which peak at some point during chick development and decline thereafter (e.g., Kitaysky, 1999). Because cort implants could not increase the physical capacity of kittiwakes to ingest food, the difference between controls and cort-implanted individuals became obvious when controls decreased their food consumption. Despite a higher rate of food intake, individuals treated with cort did not grow body mass (or skeletal elements) at a higher rate compared to controls. This suggests that cort treatments either increased energy expenditure or decreased assimilation efficiency of kittiwake chicks.

Cort during development and cognition later in life

During food shortages, parent kittiwakes are willing to increase their effort in foraging for the young in response to chick begging (Kitaysky et al., 2000, 2001b). However, depending on the severity and duration of food shortage, parent kittiwakes are not always able to fully buffer their young from fluctuations in food supply. When they cannot, chicks are exposed to chronically elevated levels of cort (Kitaysky et al., 1999, 2001a). Nevertheless, kittiwake chicks are developmentally plastic (Romano, 2000), and if foraging conditions improve (due to brood reduction and/or environmental change), they are capable of compensatory growth and might still fledge successfully (Kitaysky et al., unpublished data). The results of the current study suggest that even a moderate (in terms of magnitude and duration) elevation in cort levels has long-lasting detrimental effects on cognition of affected individuals later in life. Thus, although kittiwakes are able to overcome effects of temporal nutritional deficit on their morphological development, prolonged exposure to elevated levels of cort might compromise their fitness.

Post-fledging survival of kittiwakes might be affected by exposure to elevated levels of cort during development. Young kittiwakes are independent of their parents after fledging (Baird, 1994) and must learn quickly how to forage on their own. Successful foraging requires finding a patch of food (see below) and the actual act of capturing and handling fish. The results of this study suggest that the rate of learning/mastering a skill of handling prey might be severely compromised by exposure to elevated levels of cort during development. In particular, individuals treated with cort took approximately twice as long to master the skill of

“opening a dish” compared to controls (Fig. 4). Despite the superficial simplicity of this task, successful completion required chicks to develop the skill of pecking on the side of a lid at an angle. For instance, individuals that failed to open a transparent dish containing food failed because they hammered straight down on the lid incessantly rather than trying new ways to open it. Other individuals treated with cort during development succeeded in learning the task of opening a dish, but were still slower than controls. Thus, we do not know whether motor skills and/or memory retention were damaged by elevated cort. Regardless of the specific mechanism(s) causing inferior performance of kittiwakes in mastering the foraging technique, it is probably applicable to capture and handling of live and elusive prey by kittiwakes in the wild.

The distribution of food resources in marine environments is known to be patchy (Lack, 1968; Hunt and Schneider, 1987), which makes the learning task more difficult for young inexperienced birds. Most often, kittiwakes forage on patches of food (fish and invertebrates) concentrated within visually distinctive oceanographic features, such as fronts, eddies, and upwellings in oceanic and near-shore environments (Hunt and Schneider, 1987; Irons, 1992). Thus, the ability of kittiwakes to make associations between visual signals (water color, surface structure, topographic landmarks) and presence of food (fish and invertebrates) is essential for their foraging success. The results of this study suggest that the ability of young kittiwakes to associate visual cues with the presence of food in a choice situation might be severely compromised by exposure to elevated levels of cort during development. In particular, foraging efficiency (as determined by the number of correct choices made over the total number of correct choices available) of individuals treated with cort was two times lower than controls (Fig. 5). The decrease in foraging efficiency might increase likelihood of post-fledging mortality of kittiwakes due to starvation.

Variations in fitness of adult birds might be related to their nutritional history at the juvenile stage of life (reviewed in Metcalf and Mognahan, 2001). Recent studies of black-legged kittiwakes showed high heterogeneity in quality of first-time breeders (Cam and Monnat, 2000). In particular, Cam and Monnat demonstrated that the reproductive success and survival of within-cohort individuals are significantly positively correlated—birds that bred successfully were more likely to survive. Reproductive success of kittiwakes mostly depends on abilities of parents to provide their young with food. Based on the results of our experiments, exposure to a moderate elevation of cort during development may permanently damage the abilities of black-legged kittiwakes to learn foraging techniques. Thus, quality of adult kittiwakes as breeders and foragers might reflect their nutritional history during development.

The results of this study also suggest that a bird's abilities to solve spatial tasks were impaired by the exposure to a moderate elevation of cort during early development.

Specifically, all kittiwakes learned to use an opening to escape the enclosure, but cort-treated individuals fixated upon one solution to the spatial problem but were unable to generalize that solution to a similar problem. We believe that the abilities of animals to solve spatial tasks and rely on their previous experience when faced with novel situations are relevant to their success in foraging, nest defense, and predator avoidance.

The results of this study identified short-term benefits and long-term costs associated with an episode of experimentally elevated cort levels in captive chicks. In the future, direct fitness measures in the field are required before the phenotypic effects measured in the laboratory might be directly extrapolated to birds in the wild. We also acknowledge that cort implantation provided only an imitation of elevated secretion of steroid, and that exogenous cort could alter the endogenous activity of some other hormones (CRH, AVP, or one of the POMC products) which might be associated with the observed effects.

Where to go from here?

The facilitation of chick foraging behavior (begging and competition for food) through secretion of cort might represent an evolutionary stable signaling system that is used by parent birds to assess nutritional status of their young (Kitaysky et al., 2001b; Wingfield and Kitaysky, 2002). Therefore, one might expect that the adrenocortical response of chicks to nutritional deficit would be similar among different species of birds with dependent young. Yet, the adrenocortical responses of chicks to nutritional deficit are not uniform among different species. For instance, chicks of domestic fowl (*Gallus gallus domesticus*, Freeman et al., 1981), blue-footed boobies (Nuñez-de la Mora et al., 1996), two species of kittiwakes (Kitaysky et al., 1999, 2001a), glaucous-winged gull \times western gull hybrids (*Larus glaucescens* \times *L. occidentalis*, A.E. Edwards, unpublished data), caspian terns (*Sterna caspia*, D. Lyons, unpublished data), and common murre (Kitaysky et al., unpublished data) respond to nutritional deficit by elevating cort secretion. On the other hand, there was no increase in cort secretion in response to low nutritional condition and/or reduction in energy intake in chicks and juveniles of three species of passerines (Romero et al., 1998; Schwabl, 1999; Sims and Holberton, 2000), two species of puffins (*Fratercula* spp., Kitaysky et al., unpublished data), young domestic fowls (Rees et al., 1985), domestic turkey in vitro study by Carsia and McIlroy, 1998), and american kestrels (*Falco sparverius*, Heath and Dufty, 1998).

Why are adrenocortical responses of chicks to a nutritional challenge not uniform across species? There are three mutually nonexclusive hypotheses that address differences in HPA axis activation in response to nutritional challenges: developmental, phylogenetic, and ecological. The developmental hypothesis was proposed in observational studies by Schwabl (1999) and Sims and Holberton (2000). These

authors suggested that the HPA axis probably becomes functional later in a chick's life in altricial compared to precocial species of birds.

The observed differences in juvenile physiological traits in different species of birds might also be subject to phylogenetic constraints. For instance, in accordance with phylogenetic hypothesis, two species of kittiwakes and glaucous-winged gull \times western gull hybrids belong to the same family, *Laridae*, and adrenocortical responses of their chicks to nutritional deficit are similar. However, contrary to phylogenetic hypothesis, chicks of closely related (Friesen et al., 1996) alcids, *Alcidae*, puffins, and the common murre have opposite adrenocortical responses to a reduction in energy intake. Thus, phylogenetic hypothesis alone is unlikely to explain the observed differences in chick adrenocortical responses.

Finally, the differences in the adrenocortical response of chicks might have an ecological basis. The frequency and magnitude of unpredictable environmental perturbations resulting in food shortages vary along environmental gradients. Physiological responsiveness (or latency of response) of birds to food shortages might reflect this variability (Wingfield and Kitaysky, 2002). For instance, metabolic responses to variability in food resources and survival of chicks reflect seabirds' ecological characteristics as well as that of their prey (e.g., Kitaysky, 1999; Kitaysky and Golubova, 2000). Also, provisioning strategies of parent seabirds might either buffer their young from or further exacerbate the effects of variability of food resources on chick provisioning (e.g., Kitaysky et al., 2000). Early development and high sensitivity of the HPA axis to nutritional deficit would be expected in chicks relying on continuously available food resources, which result from low environmental stochasticity and/or high parental sensitivity to chick demands. Likewise delayed development and/or suppressed sensitivity of the HPA axis to nutritional deficit are expected in chicks routinely faced with intermittent food provisioning resulting from high fluctuations in food resources and/or low parental sensitivity to chick's demands.

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